Effects of a common insecticide on wetland communities with varying quality of leaf litter inputs☆

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ARTICLE INFO

ABSTRACT

Chemical contamination of aquatic systems often co-occurs with dramatic changes in surrounding terrestrial vegetation. Plant leaf litter serves as a crucial resource input to many freshwater systems, and changes in litter species composition can alter the attributes of freshwater communities. However, little is known how variation in litter inputs interacts with chemical contaminants. We investigated the ecological effects resulting from changes in tree leaf litter inputs to freshwater communities, and how those changes might interact with the timing of insecticide contamination. Using the common insecticide malathion, we hypothesized that inputs of nutrient-rich and labile leaf litter (e.g., elm [*Ulmus* spp.] or maple [*Acer* spp.]) would reduce the negative effects of insecticides on wetland communities relative to inputs of recalcitrant litter (e.g., oak [*Quercus* spp.]). We exposed artificial wetland communities to a factorial combination of three litter species treatments (elm, maple, and oak) and four insecticide treatments (no insecticide, small weekly doses of 10 $\mu g\,L^{-1}$, and either early or late large doses of 50 $\mu g\,L^{-1}$). Communities consisted of microbes, algae, snails, amphipods, zooplankton, and two species of tadpoles. After two months, we found that maple and elm litter generally induced greater primary and secondary production. Insecticides induced a reduction in the abundance of amphipods and some zooplankton species, and increased phytoplankton. In addition, we found interactive effects of litter species and insecticide treatments on amphibian responses, although specific effects depended on application regime. Specifically, with the addition of insecticide, elm and maple litter induced a reduction in gray tree frog survival, oak and elm litter delayed tree frog metamorphosis, and oak and maple litter reduced green frog tadpole mass. Our results suggest that attention to local forest composition, as well as the timing of pesticide application might help ameliorate the harmful effects of pesticides observed in freshwater systems.

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1. Introduction

Understanding the function of ecosystems requires attention to the activity and turnover of species, as well as the physical and chemical changes in the environment. For example, temperate ecosystems have experienced dramatic changes in species composition owing to fire suppression, logging, habitat fragmentation, and hunting of top predators (Abrams, 2003). At the same time, humans have dramatically amended the landscape to increase agricultural output leading to the increased use of pesticides

to control pest species and disease vectors (Grube et al., 2011). Despite efforts to control unwanted dispersal of pesticides, contamination of non-target communities and ecosystems remains a widespread concern (Gilliom et al., 2006). Given that such chemical contamination is co-occurring with changes in the species composition of remaining forest fragments, it is imperative that we understand how the two factors interact.

In temperate forests, variation in tree species composition results in numerous changes in terrestrial and aquatic food webs. For example, tree leaf litter serves as a prominent organic subsidy of energy and nutrients in streams and wetlands (Webster and Benfield, 1986; Wallace et al., 1997; Moore et al., 2004). The energy and nutrients within litter are released through processes that include leaching, fragmentation, and microbial enzymatic activity (Moore et al., 2004). These processes promote aquatic primary production, and consumers subsequently utilize litter fragments,

microbes, and algae as resources for growth and development (Moore et al., 2004; Holgerson et al., 2016). Interspecific variation in the chemical quality of leaf litter can lead to substantial changes in the structure and function of detrital food webs (Webster and Benfield, 1986; Leroy and Marks, 2006). For example, recalcitrant litter (e.g., litter rich in lignin and cellulose) can reduce nutrient and energy availability for consumers, but might also promote stable population sizes of primary producers and consumers (Melillo et al., 1982; Geddes, 2015). In contrast, highly labile litter substrates can rapidly leach soluble carbon and promote algal and consumer growth (Cottingham and Narayan, 2013). However, excess amounts of leached carbon can darken the water column, reduce primary productivity, increase aerobic respiration, and subsequently generate inhospitable conditions for many aquatic organisms (Stephens et al., 2013; Cottingham and Narayan, 2013; Fey et al., 2015; Stoler et al., 2016). Similarly, leaching of phenolic acids can inhibit litter decomposition, reduce rates of nutrient cycling, and directly harm consumers by hindering the functionality of cell processes (Melillo et al., 1982; Webster and Benfield, 1986; Hättenschwiler et al., 2005; Maerz et al., 2005).

Changes in the chemical composition of aquatic environments might interact with pesticide contamination. Although most systems are not contaminated at concentrations that are likely to directly harm non-target organisms, pesticides can become more toxic when combined with other chemical or biological stressors (Relyea, 2003, Relyea and Diecks, 2008). For example, subtle changes in environmental chemistry, such as variation in pH due to the addition of acidic humic compounds might increase the duration of pesticide exposure by slowing rates of chemical breakdown (Wolfe et al., 1977). Resource stress imposed by litter species of low nutritional quality might also interact antagonistically with the presence of chemical contaminants. In contrast, the presence of dissolved organic carbon from rapidly decaying litter might bind to hydrophobic pesticide contaminants and alleviate toxic effects on aquatic consumers (Benson and Long, 1991; Wershaw et al., 1969; Haitzer et al., 1998). Although no studies have explored the interaction between pesticide contamination and qualitative variation in leaf litter inputs, elucidating these potential effects would greatly improve management and mitigation efforts.

Among the myriad chemicals applied to the landscape, organophosphates accounted for 35% of all insecticides (as of 2007; Grube et al., 2011). Malathion is among the most commonly applied organophosphates in the US (Grube et al., 2011). The hydrophobic chemical works by inhibiting acetylcholinesterase and typically slowing nerve cell signal transmission. Malathion is commonly found in aquatic systems due to misuse, overspray, and aerial drift from applications that frequently occur at multiple times during a growing season (Grube et al., 2011). Consequently, non-target organisms might be subjected to prolonged insecticide exposure. Previous work has found that low concentrations of the insecticide can be highly toxic to several species of zooplankton grazers, leading to trophic cascades and systemic effects on wetland communities (Relyea and Diecks, 2008; Relyea, 2009). However, studies have also shown that malathion can serve as a source of phosphorus for nutrient-limited microbial species (Rosenberg and Alexander, 1979). In turn, this fertilization effect might have positive consequences for organisms less sensitive to insecticide exposure. Hence, it is possible for the chemical to have both detrimental and beneficial effects on different parts of the aquatic community.

In this study, we explored the interaction between malathion contamination and variation in tree leaf litter inputs. Based on previous studies, we hypothesized that the presence of nutrient-rich litter (i.e. American elm) would increase microbial, algal, and consumer growth relative to nutrient-poor labile litter (i.e. red maple) or recalcitrant litter (i.e. oak). In addition, we hypothesized that malathion contamination would result in trophic cascades in which zooplankton die, phytoplankton bloom, and periphyton declines leading to lower consumer growth. Moreover, the effects caused by malathion exposure would be most severe under conditions of repeated insecticide application. Lastly, we hypothesized that inputs of nutrient-rich litter (i.e., elm) — which can promote periphyton growth and also leach carbon compounds (e.g., tannins) that bind to hydrophobic insecticides (Haitzer et al., 1998) — would ameliorate the negative effects of insecticides on wetland communities relative to inputs of recalcitrant litter.

2. Methods

2.1. Malathion in the environment

Models of drift and atmospheric deposition indicate expected environmental concentrations (EECs) of malathion in the range of 0.6–89.8 $\mu g\ L^{-1}$ (Mastrota et al., 2010), although wetland surveys have found up to 600 $\mu g\ L^{-1}$ (California Department of Fish and Game, 1982). Based on a USEPA risk assessment on the California red-legged frog (*Rana aurora draytonii*), average EEC of malathion in water is $9 \pm 27\ \mu g\ L^{-1}$ (95% CI) for application frequencies of 2–14 d (Odenkirchen and Wente, 2007). Although the half-life of the chemical under neutral conditions is relatively short, it varies with changes in pH, with half-lives at pH of 6 and 8 equal to 26 and 2 d, respectively (Guerrant et al., 1970; Wang, 1991). Moreover, multiple applications of the insecticide typically occur in a single season; manufacturer recommendations typically suggest applying the chemical two to four times per season at 4- to 7-d intervals (Bonide Products, Inc, Oriskany, NY). Although concentrations in non-target aquatic systems have dropped slightly in recent years (Stone et al., 2014), malathion remains among the most commonly used organophosphate insecticides on the current market.

2.2. Experimental design

To test our hypotheses, we conducted an experiment in outdoor mesocosms containing a diverse community of microbes, algae, zooplankton, amphipods, snails, and amphibians. We conducted our experiment during summer 2015 at the Rensselaer Aquatic Lab in Troy, New York. Our experiment consisted of a full-factorial design including three leaf litter treatments crossed with four insecticide treatments. Leaf litter treatments consisted of three common tree species: elm (*Ulmus americana*), red maple (*Acer rubrum*), and black oak (*Quercus velutina*). Insecticide treatments consisted of a no-insecticide control, 10 μg malathion L^{-1} delivered weekly, 50 μg malathion L^{-1} delivered once at the beginning of the experiment, and 50 μg malathion L^{-1} delivered once three wks after the start of the experiment (treatments are henceforth referred to as 10-weekly, 50-early, and 50-late, respectively). We selected these insecticide concentrations and application frequencies to correspond with a range of environmentally relevant values and to simulate realistic application regimes for agricultural use (Relyea and Diecks, 2008). We replicated each of the 12 treatment combinations four times for a total of 48 experimental units. Experimental units consisted of 900-L, black, polyethylene cylindrical tanks. We covered each mesocosm with a 60% shade cloth to prevent the escape or entry of any organism and to simulate moderate canopy cover (Schiesari, 2006).

We filled tanks with 550 L of chlorinated tap water between 1 June and 3 June, and allowed chlorine to off-gas before adding leaf litter. On 12 June, we added leaf litter to all tanks. We collected leaf litter from the ground of local forests during spring 2015 and allowed all litter to air-dry prior to adding it to the experiment. The